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Treatment of Experimental Paralyses with Tetraethyl Monothiopyrophosphate

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In the present communication I shall report the experimental data secured in two laboratories.

In February-April 1955 in the Department of Pharmacology of Minsk Medical Institute under the direction of Prof. K. S. Shadurskii there was studied by me the effect of tetraethyl monothiopyrophosphate (TETP) in pharmacotherapy of peripheral weak paralyses of traumatic origin. In September-November 1955 in the Department of Pharmacology of the Institute of Experimental Medicine and Academy of Medical Sciences USSR in Leningrad, under the direction of the Member of the Academy Prof. S. V. Anichkov, there were made studies of pharmacotherapy of central paralyses of neurovirus etiology.

Having discovered in the previous work that TETP produces a stimulating action of the N-cholinoreceptors of the central nervous system and the skeletal musculature, and also considering the literature data on the use of some substances with anticholinesterasic activity in treatment of weak (or flaccid) paralyses in clinics, we set out to study the action of TETP in the treatment of peripheral paralyses on white mice.

Traumatic damage of the sciatic nerve was performed according to the method developed by M. A. Rozin in N. V. Lazarev's laboratory. Each experimental group consisted of twenty mice (thus 400 total). The degree of damage of the sciatic nerve was such that in the control mice the tone of the distal muscles determined at the distance of 1-5 and 2-4 fingers was restored only after 30 days after the application of the Dieffenbach clamp. The aqueous solution of TETP was introduced subcutaneously and in some experiments it was administered into the stomach and at the site of damage in the

dosage of 0.06 and 0.18 mg/kg. The introduction of TETP was done both prophylactically before the operation and after various intervals following the latter.

It was established by these experiments that the course of paralyses originating as the result of traumatic damage to the sciatic nerve reproduces the clinical form of flaccid paralyses with loss of sensitivity, trophic disturbances, reactions of partial reformation and loss of ligament reflexes.

In mice which were left without treatment (fig.1, experiment 1) or after administration subcutaneously of 0.5 ml. of isotonic solution of table salt, the cure set in slowly and only after 27-30 days following the damage did the tone of the distal muscles reach the original figures. Here in some 25% of the cases there were observed trophic disturbances which led to necrosis of fingers and loss of weight.

After subcutaneous administration of TETP in the dosage of 0.06 mg/kg on the third day following the damage the tone of the distal muscles in the foot with the damaged nerve was noticeably increased. Subsequent double administration of TETP on the 15th day caused a complete restoration of the tone (experiment 2). The pain sensitivity was restored along with ligament reflexes and the trophic disturbances occurred only in singular cases.

Operation

TETP 0.06 mg/kg

TETP 0.18 mg/kg

TETP 0.18 mg/kg

TETP 0.18 mg/kg

Days

Fig.1. Treatment of paralyses with tetraethyl monothiopyrophosphate.

It follows from this experiment that the cure occurred 2.3 times for rapidly under the action of TETP in the indicated dosage in comparison with the control.

After increase of the dose by 3 times in the following group of mice (experiment 3) a complete restoration of the foot function with the damaged nerve took place on the 10th day, or three times faster than the control.

In subsequent groups (experiments 4 and 5) a daily administration of TETP in the dose of 0.18 mg/kg and especially 5 hours after the damage assured already on the 4th day a considerable increase of tone of the distal muscles.

From this it follows that the most rapid use of TETP after a damage creates favorable conditions for clinical cure.

Following these studies we introduced in the subsequent groups (fig. 2, experiments 6, 7 and 8) TETP prophylactically eight days before the operation, using a daily dose of 0.18 mg/kg.

It is evident from these figures that the prophylactic administration of TETP increased the muscle tone even in unoperated mice (experiment 6); after the damage to the sciatic nerve the distance between fingers did not fall to the lowest figures (4-3 mm) but remained at the higher levels (5.2-4.8 mm). In this experiment in which a subsequent administration of TETP was not performed a complete cure took place on the 6th day after the operation, i.e. five times more rapidly than in control mice (exp.6).

It follows from this experiment to all appearances that the prophylactic administration of TETP in diseases or manipulations which may lead to the development of paralyses is desirable.

Operation

TETP 0.18 mg/kg

TETP 0.18 mg/kg

TETP 0.18 mg/kg internally

Days

Fig.2.

In experiment 7, along with the prophylactic administration, the substance was also administered during eight days daily in the dose of 0.18 mg/kg.

By the fourth day it was possible to note the considerable restoration of the tone, with complete cure in 12 days.

It follows from this experiemnt that a frequent administration of the substance does not have considerable advantages over the technique of its administration every other day.

After administration of the substance into the stomach (experiment 8) in the same dosage we were able to note that cure in the experimental animals occurred in the same period of time as after subcutaneous administration.

It follows from this that in a clinical study of the substance one may successfully use the oral method of administration, and at the same time it was established that in the oral method of administration of organophosphorus compounds their toxicity is decreased by 7-10 fold.

Having obtained such results we compared them with the results of treatment by other substances used for therapy of flaccid paralyses. We ran experiments with Dibazol and obtained the data which were analogous to those in the literature.

As can be seen in fig.3 (experiments 9 and 10) Dibazol taken in rather large doses of 10 mg/kg in the first period almost fails to give a therapeutic effect, but used in the second, restorative period of the clinical course of the paralyses it caused in the same dosage an exceptional effect. In the latter case cure took place on the third day after use of Dibazol.

In combined treatment with Dibazol and TETP it was possible to note that during the first period of the clinical course of the paralyses (exp.11) the restoration of the tone of the distal muscles proceeded energetically and on the third day it was almost complete. Complete cure took place in 10-12 days, i.e. the same period as with TETP alone.

Dibazol 10 mg/kg

Dibazol 10 mg/kg

Dibazol + TETP

Dibazol + TETP

Dibazol + TETP

Phenadon 1 mg/kg + TETP 0.18 mg/kg

Days

Fig.3.

It follows from the above that the restoration of the tone took place under the influence of TETP and that Dibazol displacyed no noticeable effect.

From the comparison of the action of Dibazol and TETP used together during the second period of the clinical course of the paralyses (exp.12) it is evident that a single administration of TETP and Dibazol produces a complete cure of the tone 17 times more rapidly than in the control.

For clarification of the question as to whether or not TETP possesses under our conditions any cumulative effects and whether or not it causes an overstimulation and exhaustion of the nervous system. we set up some experiments with prolonged administration of the substance. After daily administration of TETP and Dibazol over twenty days (exp.13) we failed to note the phenomena of overstimulation or exhaustion of the nervous system. Cure occurred after 11 days.

From the above one may conclude that the physiological mechanism of the basis of pharmacological activity of the substance in these doses is not beyond the functional threshold of the nervous system.

In connection with the fact that the restoration of the tone of the

distal muscles to the original level may be considerably hindered by pain originating in the nerve damage and to some degree in the muscle, we set some experiments with combined therapy of paralyses by means of Phenadon in the dose of 1 mg/kg and TETP in the dose of 0.18 mg/kg. It was shown by these experiments that the restoration of the tone of the distal muscles took place on the third day of the treatment. Complete restoration of the foot function was noted on the 13th day.

It should be noted especially that in experiments in which TETP was used in combination with Dibazol or Phenadon, trophic disturbances were not seen in a single case in the operated foot. This positive effect of the indicated combination in treatment of flaccid paralyses which are accompanied by trophic disturbances should attract the attention of the clinicists.

TETP was also studied during the period of residual phenomena of the clinical course of the paralyses, i.e. after one and two months following the traumatic damage of the sciatic nerve. It was shown that TETP was sufficiently effective for treatment of the residual phenomena after paralyses. The greatest therapeutic effect in this case was observed in mice in which the lowered muscle tone stayed in the low range of figures throughout the month.

The stimulating effect of TETP on the N-cholinoreactive structures of the central nervous system allows one to consider that this substance may also give a favorable effect on the course of central paralyses (poliolyelytic, lead, etc.) which are the result of afflictions of the appropriate regions of the central nervous system.

A further study of the action of TETP was made on paralyses of central origin following the suggestion by Prof. S. V. Anichkov. The high lipoidophilic nature of organophosphorus compounds, including TETP, gave one reason to believe that this substance should possess not only the peripheral predominantly toxic action, but was what most important - a central action.

Results of experiments with the use of tetraethyl monothiopyrophosphate (TETP) in the pharmacotherapy of paralyses of neurovirus etiology.

Virus	LD50	Number of animals	Number of groups in experiment	Paths of administration of the substance	Results of therapy (ratio of recovered to dead animals) After total death in control	Results of therapy (ratio of recovered to dead animals) At end of experiment	Duration of observations Days	Remarks
Virus of spontaneous mouse encephalomyelitis (Taylor virus) strain K3	1,000,000	Guinea pigs,20	5	Prophylsubcut.	2:0	1:0	16	In one of recovered pigs, the tone of distal muscles was restored
				Proph. intragast.	2:0	0:0		
				Subcut.treatment	2:0	0:0		
				Intragestr.treat.	0:0	0:0		
				Virus control	Dead in 8 days			
Same virus	100,000	Guinea pigs 28	4	Virus control	Dead in 14 days		30	In 7 of the recovered animals, no paralysis developed in four.
				Proph.subcut.	7:0	7:0		
				Control,NaCN	4:0	1:0		
				Proph.subcut. + NaCN	2:0	0:0		
Lansing strain of poliomyelitis virus	100	White mice,50	5	Virus control	Dead in 11 days		15	
				Proph.subcut.	1:0	1:0		
				Subcut.therapy + O_2	3:0	3:0		
Virus of two-wave Leningrad tick meningoencephalitis	100 and 100,000	White mice 100	-10	Proph.subcut.	6:2	6:2	13	Virus 10^{-4}
				ditto	2:0	2:0		virus 10^{-2}
				Proph.gastr.	2:2	2:2		virus 10^{-4}
				ditto	1:0	1:0		virus 10^{-2}
				Subcut.therap.	1:0	1:0		virus 10^{-2}
				Gastr.therapy	1:0	1:0		virus 10^{-2}
				Virus control 10^{-4}	Two alive			
				ditto 10^{-2}	Dead in 12 days			
				TETP control,0.13 mg/kg	All alive			
				Total control	All alive			

Continued table

Herpes virus adapted to nerve tissue	3 and 300	White mice	80	8	Subcut. prophyl. + virus 10^{-3}	5:0	0:0	Estimated virus titre LD50 1:318200
					ditto + virus 10^{-5}	9:8	3:4	
					ditto +NaCN +virus 10^{-3}	3:0	0:0	
					ditto +NaCN+virus 10^{-5}	4:8	1:4	14
					Control of virus 10^{-3}	Dead in 10 days		
					control of virus 10^{-5}	Four alive		
					NaCN+virus 10^{-3}	5:0	0:0	
					NaCN + virus 10^{-5}	7:8	1:4	

Table 2.

Results (comparison of mortality) of use of TETP in guinea pigs infected with the virus of spontaneous mouse encephalomyelitis (Taylor virus, strain K3; LD50 100000 for mice)

Experimental group	Number of animals	Day after infection: 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	30th	∞
Virus control	7	7	7	7	7	7/2	7/3	6/3	5/5	3/3	3/3	3/3	2/2	2/2	----	--	
Prophylact.TETP	7	7	7	7	7	7	7	7	7	7	7	7/3	7/3	7/3		7/2	
NaCN control	7	7	7	7	7	7	7	7/3	7/4	7/4	7/4	7/5	5/5	5/5	4/4	1/1	
Prophyl.TETP+NaCN	7	7	7	7	7	7	3/1	3/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	--	

It is specifically this that advantageously separates TETP from other anti-cholinesterasic substances which do not possess the ability to bind themselves with the lipoids of the nerve tissue. We found no studies in the literature on this problem. The oxygen analog, TEPP is being used abroad for clinical purposes in muscular weakness.

As an experimental model for central paralyses we selected the infectious paralyses which develop in animals after infection by the virus of human poliomyelitis (Lansing strain), by mouse encephalomyelitis (Taylor strain K3), by two-wave Leningrad tick meningoencephalitis, and by herpes virus (Herpes simplex) adapted to the berve tissue.

This study was necessary as well for the reason that the site of localization of the pathological process in many neurovirus ailments and the site of application of action of the test compound coincide (the frontal process of the spinal cord and the region of respiratory center in the medulla oblongata).

It was of interest to clarify not only the origination and the development of paralyses, the state of the tone of distal muscles of paralyzed extremities, but also the behavior of the virus, its reproduction in the biochemically and functionally altered nerve cell under the action of TETP.

The cytopathogenic action, alteration of the titre of the virus-neutralizing and complement-binding antibodies were studied on Syrian rats, guinea pigs, white mice with intrabrain and intraperitoneal infections, as well as with developing hen embryos and in cultures of the tissues of cancer tumors (Hell strain). In addition, observations were made on the temperature, the weight and general state of the animals. The results of the experiments are shown in the table. The work was completed in collaboration with the Virusology Department of the Institute of Experimental Medicine, Academy of Medical Sciences USSR, by scientific workers L.M.Kurnosova and V.I.Il'enko.

In the first experiment after infection of guinea pigs-sucklings, having

body weight of 50-100 grams, with the virus of spontaneous encephalomye[illegible] (virus of Taylor, strain K3) in the amount of 1000000 LD50 the control ani[illegible] died completely on the 8th day with severe symptoms of paralysis of the respiratory center and paralysis of front or hind legs.

Animals which were given TETP in the dosage of 0.13 mg/kg were not all dead at that time. In the three groups there were left two guinea pigs ea[illegible] In one group TETP was administered prophylactically three days before the infection, while in other two groups it was given prophylactically into the stomach and in subcutaneous therapy. In all six animals remained alive from the 16, after the death of the controls. At the end of the experiment on the 16th day there was left alive only one guinea pig from the group of animals which had been given TETP subcutaneously prophylactically. Paralysis development was nearly the same in the animals of all five groups. In one of the recovered guinea pigs the tone of distal muscles was restored.

This experiment was then repeated with the only difference being that for the test we took animals which were older having body weight of 150-300 grams, and the amount of vurus was reduced by 10 fold (100000 LD50). TETP in the same dose was administered not once but two times a day and the administration was begun not three, but eight days before the infection by the same virus (Taylor virus, strain K3).

As the result of the experiment in the control group of the animals there appeared paralyses on the fifth day after infection in two animals of the seven in the group, while on the seventh day there began the death of animals with clearly shown phenomena of paralysis of the respiratory center. By the 14th day after infection all control animals died. In all the animals without exception there developed paralyses of the front or hind legs.

In the group of animals receiving TETP both prophylactically as well as after the infection the introduction of the virus did not cause such severe phenomena and the animals bore the event relatively easily. Of the seven recovered animals paralyses failed to develop at all in four, while

In three animals the paralysis arose on the 11th day, i.e. six days later than in control. On the 20th day after infection the paralysis of a left front leg in one of the animals passed away and the leg function was restored completely.

The use in this experiment of sodium cyanide as one of the most powerful enzymic poisons either alone or combined with TETP gave an insignificant result.

At the end of the experiment after thirty days after infection the recovered animals had a healthy appearance and gained on the average 30 grams in weight.

It follows from this experiment that the prophylactic use of TETP produces a considerable therapeutic effect after infection by a relatively large dose of the virus and protects the animals from death, lowering the incidence of resulting paralyses by approximately a factor of two.

One may think that TETP prevents the development of paralysis not only in the motor analyzer but also in the region of the respiratory center, if the death of the animals is regarded as the result of paralysis of mainly the respiratory center.

The protective action of TETP in animals against the virus of human poliomyelitis, adapted to mice (Lansing strain), against the virus of two-wave Leningrad took meningoencephalitis and against the herpes virus adapted to nerve tissue, is expressed to a lesser degree - 50-30% in comparison with the control (4:0 of 200, 13:4 of 100, 21:16 of 80, respectively, after total death of the controls). Here in the mice which recovered after the infection by the virus of human poliomyelitis no paralyses developed.

It follows from these experiments that the degree of protection by TETP against various neuroviruses is not the same. The latter fact can be explained by the possible difference of viruses in the biochemical sense, and also in part by the low degree of perfection of the experimental models with these viruses for pharmacological studies. The experiments had to be run under extremely unfavorable conditions for development of the therapeutic action

TETP. Thus for example the infection by the virus was done intracerebr... by a path which does not occur under natural conditions. Here the virus was taken in very large doses of up to 1000000 LD50.

Having obtained such data we decided to clarify the direct action of TETP on the virus.

For this purpose the herpes virus in various dilutions was mixed with an equal volume of a solution of TETP in final concentration of 1:100000. Then the mixture was introduced into the amnyotic cavity of developing hen embryos of 10 day age. After 48 hours the entire amnyotic fluid was used to infect white mice in which the virus titration was run.

As the result of the experiment it appeared that the death of both the control mice and the mice infected by a mixture of TETP+virus was the same with appropriate dilutions of the virus.

In connection with this the question appears as to conclusion that TETP does not have a direct harmful effect on the herpes virus. In this experiment we also failed to note any virus-static action of TETP.

Protective action of TETP, one must assume, is conditioned by its direct action on the nerve cell, the biochemistry and the functional state of which are altered under the influence of TETP. The invading virus apparently does not find, under the conditions of such an altered cell, the favorable conditions for its development. Not having its own enzymes, the virus does not receive food or energetic substances and is weakened as a result of this. Besides this weakening of the virus, there is also the aid from the growth of the titer of the virus-neutralizing antibodies. The protective reaction of the organism, displayed by the rise of body temperature, along with other factors assures a destructive action on the virus. The thus developed conditions as the result of interaction of the virus with the organism of the animal which had received TETP resemble in many ways the vaccination of an animal by a weakened virus culture. The difference consists only of the fact that in our experiments this weakening occurs in the nerve cell itself.

On the basis of our experiments it is possible to suppose the [illegible] a new path for interaction on small neuroviruses.

Among organophosphorus compounds, thus, there may be found even more active substances which may be less toxic than TETP.

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Remarks.

B.A.Arbuzov (Kazan Section, Academy of Sciences USSR)

It is pleasant for me to hear that organophosphorus compounds synthesized in the Kazan Section are finding more and more new areas of application. We can satisfy the requests of pharmacologists by supplying to them in needed quantities Pirofos and other organophosphorus compounds similar to it in chemical structure. The contact between the chemists and the pharmacologists will aid a rationally directed synthesis.

U.Sh.Akhmerov (V.I.Lenin State Institute for Improvement of Medical Personnel, Kazan).

The report by M.M.Lenkevich is very interesting from the viewpoint of prospective use of TETP in clinics. The question exists as to the action of TETP on the organism during the acute and the paralytic periods. The problem arises: does the cure take place through the action of the substance on the virus or on the nerve cell?

As it is known, Dibazol is effective only during the paralytic period, therefore the mechanism of action of TETP is different. From the report by M.M.Lenkevich it follows that TETP does not permit the action of the virus on the nerve cell. In our clinic we observed two cases of ascending paralysis. By using an injection of proserine already at the bulbar phenomena of paralysis, the latter was cancelled and the patients recovered. Proserine gave

improvement of the trophism of the nerve cells and thus raised the resistance to the toxin. The most rapid testing of TETP in clinics is desirable.

N.I.Vylegzhanin (V.I.Lenin State Institute for Improvement of Medical Personnel, Kazan).

In the summaries of M.M.Lenkevich report there is stated that " the protective action of TETP apparently is caused by its direct action on the nerve cell. The invading virus does not find in such an altered cell the favorable conditions for its development. The weakening of the virus is aided by the growth of the titer of virus-neutralizing antibodies". The mechanism of action of TETP in poliomyelitis is not clear. Concerning which action does the author speak: etiotropic or pathogenic?

In connection with this question I wish to recall the work on the plague by N.N.Zhukov-Verezhnikov. He showed that is one gives the possibility to the patient or infected animal to withstand the period of plague bacillemia, which is the most dangerous during the development of the disease, that the developing and intense elaboration of specific antibodies leads to a rapid destruction of the plague bacilli and recovery results. It seems to me that the substance of TETP type acts pathogenetically,rather creating conditions for the nerve cells, infected by the virus, to enable them to withstand the destructive action of the pathological irritant.

For development of the processes of immunological protection of the organism some period of time is always needed. It is this period that is so dangerous to the life of the sick organism. In order to speak of vaccination with a weakened live virus culture under such conditions, it is necessary to study the state of the virus in the organism on which TETP had acted.

M.Ya.Mikhel'son (1st I.P.Pavlov Medical Institute,Leningrad)

The report by M.M.Lenkevich is a proof of the victory of the I.P.Pavlov method- the method of experimental therapy. In connection with this report I recall involuntarily the experiments made in N.V.Lazarev's laboratory when Dibazol was being tested for treatment of aftereffects of polio.

[illegible]eriments by M.M.Lenkevich there is being discussed the problem about [illegible]ation of the substance on virus reproduction, on its viability in the organism of a warmblooded animal. This completely new action of TETP is different from the action of other anticholinesterasic compounds.

M.M.Lenkevich expressed the supposition about the action of TETP on all esterases, but in a test-tube TETP does not act on the virus; hence, the mechanism is different. For this purpose it is very interesting to run contr experiments with eserine and proserine which, as it is known, do not act on aliesterases. The treatment of polio is one of the most real problems. This alone speaks of the value of the present work. We wish success to M.M.Lenkevi in his interesting creative researches. Possibly among organophosphorus compounds it will be possible to discover even more effective and more suitable substances for clinical use than TETP.

I.D.Neklesova (Kazan Section, Academy of Sciences USSR)

Data reported by M.M.Lenkevich are very interesting. One more area is opened for application of organophosphorus compounds. I wish to direct [illegible] attention of the speaker, in connection with the proposed use of TETP in clinics, to the cumulative properties of TETP and the rapidity of hydrolysis of it in aqueous solutions. It is evident from the report that control guinea pigs did not die after daily administration of the substance in amount of 0.18 mg/kg over 18 days. It seems to me that such results cannot be obtained with a pure and unhydrolyzed specimen of TETP. Evidently M.M.Lenkevich failed to consider the possible hydrolysis of TETP and administered, therefore, a very much smaller dose. These properties of the compound must be considered in later work, in order to avoid obtaining unexpected and undesirable results.

Yu.S.Kagan (Kiev Institute for Labor Hygiene and Occupational Diseases)

M.M.Lenkevich gave new and original facts. The problem arises: does TETP alone possess these properties or is this true of others among organophosphor compounds? The activity of cholinesterase must be examined which will permit one to judge the cumulative effects of TETP.

...g word by M.M.Lenkevich

...known from the work of Japanese workers that in infection ... the amount of true cholinesterase is considerably ... We may think that the paralysis of the motor cells of ... of the spinal cord and the respiratory center occurs ... in these nerve organizations the secretion of acetylcholine ... even in the moment of statu nascendi. Losing thereby ... component which maintains the trophism of the nerve cells (... the latter die, which fact is confirmed by a series of histo... Hence the therapeutic action of TETP is clear. Introduced ... or in the moment of infection of the animals by the virus ... activates cholinesterase and thereby protects acetylch... of the nerve cell against the virus attack. We did ... cumulative effects of TETP.

...ing for the purposes of economy a day-old solutions, we ... partly hydrolyzed substance (at 32° in a day the hydroly... ...). However, the thus obtained positive therapeutic effect ... fact that products of partial hydrolysis are also useful to ... that it would be interesting to run special experiments on ... men of TETP and to clarify the action of the intact molecule of TETP ... products of appropriate transformations.
